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Organophosphate Pesticide (Methyl Parathion) Degrading Bacteria Isolated from Rhizospheric Soil of Selected Plants and Optimization of Growth Conditions for Degradation

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ABSTRACT

The present investigation dealt with the isolation of Methyl Parathion degrading microorganisms from rhizospheric soil of selected plants. The isolates were biochemically identified as *Pseudomonas aeruginosa*, *Bacillus megaterium* and *Staphylococcus aureus*. These microorganisms could degrade Methyl Parathion upto 350 µg/ml concentration. Among all isolates the highest incidence was of *Bacillus megaterium* followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The optimum pH for the growth of Methyl Parathion degrading isolates was observed as pH 7.0 and optimum temperature for their growth was observed as 30˚C. The investigation would be of great help in order to degrade Methyl Parathion. In future, these species can be used along with a consortium of other bacteria for proper degradation of the pesticide. Also, this could be genetically engineered to yield a strain which would degrade Methyl Parathion on a large scale.

Key words:

P. aeruginosa, *B. megaterium*, *S. aureus*, Methyl Parathion, Pesticide, Rhizospheric soil

INTRODUCTION

India is an agriculture based country. About 60-70% population is dependent on agriculture. The demand for agriculture crops is increasing day by day due to rapidly increasingly population. Hence, there is need for a huge increase in quantity of agricultural produce and improvement in its quality. To meet these objectives; agrochemicals like insecticides, fungicides, pesticides and herbicides, use of better quality seeds are being used. About 30% of agricultural produce is lost due to pests. Hence, the use of pesticides has become indispensable in agriculture **[Ghosh *et al.,* 2010]**.

Pesticides are organic compounds manufactured and used for pest control. When pesticides are dispersed in the environment, they become pollutants, with ecological effects that require remediation. Environmental Pollution is caused by both excessive and continuous use of pesticides, and begins when these compounds enter environment by various means (accident spills, direct application, residues from cleaning of containers, state of equipments used and methods used to apply the products**) [Ortiz-Hernandez and Sanchez-Salinas, 2010].**

At present there are more than 10,000 different pesticides **[Bindhya *et al.,* 2009].** Residues of applied pesticides stay in the environment as in soil, in free and bound form and can also enter the ground and surface water compartments. At high temperature, pesticides rapidly volatilize into atmosphere and ultimately sink into aquatic ecosystem [4]. Pesticides contamination as a result of agricultural and industrial activities poses serious threats to environment and indeed to human life [**Hashmi *et al.,* 2009; Schnoor, 1992 and Ritmann *et al.,* 1988**].

An important way to avoid ecological damages and human health problems caused by the presence of pesticides is to reduce their concentrations in the environment, precluding either lixiviation to ground water or possible incorporation to natural food chains **[Ortiz-Hernandez et al., 2001].**

Organophosphorus (OP) pesticides such as Parathion, Methyl Parathion and Methamidophos are a group of highly toxic agricultural chemicals widely used in plant protection **[Pakala *et al.,* 2006**]. Because these compounds are potent inhibitors of acetylcholinesterase and have 50% lethal dose values (in rats) of as low as 4 to 13 mg/kg of body weight (Parathion) and 14 to 24 mg/kg of body weight (Methyl Parathion) **[Gains, 1969; Chaudhary *et al.,* 2010].** Organophosphate pesticides inhibit the activity of both, the cholinesterase (ChE) enzymes in red blood cells and serum-ChE resulting in the cholinergic features of Organophosphate toxicity **[Jaga, and Dharmani, 2003, Andleeb, and Qazi, 2007].**

Organophosphates may also cause delayed neurotoxic effects which are not due to acetylcholinesterase inhibition. In the presence of Organophosphates, these enzymes are phophorylated and inactivated. Once 80% of the enzyme is inactivated, usually within four days of exposure, potentially lethal symptoms can be observed, including neck muscle weakness, diarrohea and respiratory depression [**Ortiz-Hernandez and Sanchez-Salinas, 2010, Grimsley *et al.,* 1998].** Organophosphates contain three phosphoester linkages and are hence often termed as phosphotriesters. In general, only hydrolysis of one of the phosphoester bonds can reduce significantly the toxicity of Organophosphates. Organophosphorus hydrolase (OPH, EC 3.1.8.1) can specifically hydrolyze the phosphoester bonds of Organophosphates and reduce their toxicity **[Ningfeng *et al.,* 2004].** Organophosphate pesticides such as Parathion and Methyl Parathion have been used extensively as agriculture and domestic pesticides included in insecticides, fungicides and herbicides [**Liu et al., 2005**]. Methyl Parathion is a contact and ingestion Organophosphate insecticide, having P=S bond. Due to its low persistence in the environment it is used mainly in agriculture [**[Ortiz-Hernandez *et al.,* 2001].**]. p-Nitrophenol is the major metabolite of Methyl Parathion and it has moderate toxicity after single oral or dermal exposure. It is important to note that PNP is very persistant, toxic and inhibits microbial growth in natural aquatic systems [**Charoensri *et al*., 2001**]. p-Nitrophenol (PNP) is one of the major hydrolytic products generated when the Organophosphate pesticides Parathion and Methyl Parathion are subjected to microbial degradation[**Ritmann *et al.,* 1988**]. Enzymatic hydrolysis of Parathion and Methyl Parathion reduces the toxicity by nearly 120- fold and leads to the formation of PNP and Diethylphosphate Acid **[Liu *et al.,* 2005].** Identical *opd* genes coding for OPH were found in two soil

microorganisms; *Pseudomonas diminuta* **[Ortiz-Hernandez and Sanchez-Salinas, 2010, Charoensri *et al*., 2001**] and *Flavobacterium sp* [**Ortiz-Hernandez and Sanchez-Salinas, 2010, Cui, 2001].** Another gene with identical function is *mpd*, first isolated from Methyl Parathion degrading *Plesiomonas sp.*, but it showed no homology to the known *opd* genes **[Cui, 2001, Lin *et al.,* 2008]**

The purpose of this study was to examine the ability of rhizospheric microorganisms that could degrade Methyl Parathion and to investigate the optimized degradation potential of the organisms.

MATERIALS AND METHODS

Chemicals

Commercial grade Methyl Parathion (MP).

Media used for growth of Methyl Parathion degrading organisms

Nutrient Agar media (NA) contained the following ingredients (in grams per liter): Peptone, 5.0; Beef Extract, 3.0; NaCl, 5.0; Agar, 18.0. The pH value was maintained to 7.0±0.2 and then the medium was autoclaved. Trypticase Soy broth (TS) contained the following ingredients (in grams per litre): Trypticase (animal peptone), 15.0; Phytone (soy peptone), 5.0 and NaCl, 5.0. The pH was maintained to 7.3±0.2 and it was autoclaved.

Sample collection

Soil samples were collected from MP treated Agricultural sites at Research farm, SHIATS, Allahabad; Commercial farm, Jhunsi, Allahabad; and Guava Orchard, SHIATS, Allahabad from Cabbage, Tomato and Guava rhizosphere respectively *via* aseptic technique using presterilized screw cap glass bottles or polythene bags. These soil samples were processed on the day of collection by mixing 10 grams of each soil sample in 100 ml autoclaved water and keeping them in orbital shaker at 100 rpm for 24 hours at room temperature.

Isolation of Methyl Parathion degrading bacteria

The cultures capable of degrading Methyl Parathion were isolated from soil using enrichment technique, with varying concentration of Methyl Parathion in the medium. Wet unseived soil (2-5g) from agricultural site was inoculated into 250 ml of distilled water in 500 ml flasks containing 100-350 µg/ml Methyl Parathion. The flasks were incubated on a shaker operating at 240 r.p.m for four days at ambient temperature (25°C). Growth of bacterial cultures was determined by viable cell enumeration at 24, 48, 72 and 96 hours, for which 1 ml of sample from the flasks containing different concentrations of Methyl Parathion was drawn at regular intervals and serial dilution upto 10^{-4} was performed using 9 ml sterile blank. Appropriate sample was plated on Nutrient Agar medium followed by incubation at 35°C for 24-48 hours. After incubation viable colonies were counted to express the growth of bacteria supplemented with Methyl Parathion in medium. Growth was expressed in terms of cfu/ml.

Individual colonies were subcultured onto Nutrient Agar plates containing Methyl Parathion until pure cultures were obtained. Bacterial isolates that could grow at relatively high concentration of pesticide were subjected to morphological, cultural and biochemical tests. The potential of the isolated strain to utilize other pesticides for their growth was determined. Pure cultures were maintained on Nutrient Agar slants, subcultured regularly at 15 days interval on the same media and incubated at 35°C for 24 hours and stored at 4°C in refrigerator.

Identification of the isolates

The strains which degraded Methyl Parathion at different concentrations were identified by colony characteristics, growth, The isolated organisms were characterized morphologically by gram's staining and biochemical characterization which included tests like:

Carbohydrate fermentation test, Oxidase test, Catalase test, Coagulase test, Hugh and leifson's oxidation fermentation test, Arginine hydrolysis test, Urease test, Nitrate reduction test, Indole production test, Methyl red test, Voges-Proskauer test, Starch hydrolysis test, Citrate utilization test, Gelatin liquefaction test, Triple sugar iron test, Motility test

Optimization of growth conditions of Methyl Parathion degrading isolates.

Trpticase soy broth supplemented with different concentrations of Methyl Parathion (100, 150, 200, 250, 300 and 350 µg/ml) was used to determine the optimum temperature and pH for the Methyl Parathion degrading isolates.

Trypticase Soy broth was inoculated with 1 ml of 18 hrs old bacterial cell suspension at 10^{-4}. It was expressed on the basis of log cfu/ml.

Effect of different incubation temperatures on growth of Methyl Parathion degrading isolates

To determine the optimum temperature, Trypticase Soy broth containing different concentrations of Methyl Parathion was inoculated with the test organism and incubated for 24-48 hrs. After incubation the tubes were serially diluted upto 10^{-4} dilution. Appropriate bacterial sample was plated on Nutrient Agar medium followed by incubation at 10˚ C, 20˚ C, 30˚ C, 40˚ C and 50˚ C for 24-48 h. After incubation viable colonies were counted to express the growth of bacteria supplemented with Methyl Parathion.

Effect of pH on growth of Methyl Parathion isolates.

To determine the optimum pH, Trypticase Soy broth was prepared at different pH of 3, 4, 5, 6, 7, 8 and 9 supplemented with different concentrations of Methyl Parathion. The tubes were inoculated with the test organism and incubated for 24-48 h. After incubation, the tubes were serially diluted upto 10^{-4} dilution. Appropriate inoculum was plated on Nutrient Agar medium followed by incubation at 37˚ C for 24-48 h. After incubation viable colonies were counted to express the growth of bacteria supplemented with Methyl Parathion in terms of 10^4 cfu/ml.

RESULTS

Isolation and enumeration of Methyl Parathion degrading isolates

The rhizospheric soil of Cabbage, Tomato and Guava treated previously with Methyl Parathion were used to isolate the Methyl Parathion degrading microorganisms in the present study. A total of three morphologically different microorganisms capable of utilizing Methyl Parathion even at 350µg/ml concentration were isolated. A preliminary classification based on the morphology of the isolates revealed that the Methyl Parathion degrading organisms belong to the group of bacteria. The three bacterial isolates were gram negative and gram positive rods and cocci. These three bacterial isolates were identified according to the morphological, cultural and biochemical characteristics as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus megaterium*. Total bacterial load was enumerated at different time intervals and different concentrations of Methyl Parathion *i.e* 24h, 48h, 72h, 96h and 100µg/ml-350µg/ml respectively. It was observed that the bacterial growth decreased with the increase in pesticide concentration as well as time intervals. However the bacterial growth was observed maximum at 100µg/ml concentration of Methyl Parathion and followed a decreasing pattern upto 350µg/ml concentration of Methyl Parathion. Among the isolates that could degrade Methyl Parathion at different concentrations, *Bacillus megaterium* had the highest incidence i.e 49% while other isolates *i.e Psedomonas aeruginosa* and *Staphylococcus aureus* showed less incidence *i.e* 39% and 12%. Out of total 109 isolates which were found positive for Methyl Parathion degradation from rhizospheric soil of Cabbage, distribution of *B. megaterium* was highest *i.e* 62(57%), followed by *P. aeruginosa* 34(31.1%) and *S. aureus* 13(12%). Among the 92 isolates from rhizospheric soil of Tomato, maximum distribution was of *P. aeruginosa i.e* 54(58.6%) followed by *B. megaterium* 28 (30.4%) and *S.aureus* 10(11%). While, among 105 isolates which degraded Methyl Parathion from rhizospheric soil of Guava, maximum distribution was of *B. megaterium i.e* 59(56.1%) followed by *P. aeruginosa* 31(29.5%) and *S. aureus* 15(14.2%).

Table 1 Bacterial population at 10^4 cfu/ml in soil from rhizosphere of selected plants amended with different concentration of Methyl Parathion at periodic time intervals

Concentration of Methyl Parathion (μg/ml)	Time (h)			
	24	48	72	96
Cabbage				
100	**221.13±27.64**	195.9±27.13	161.8±37.34	134.06±25.87
150	144.4±30.75	128.26±27.47	94.73±33.81	68.86±32.87
200	128.6±16.12	105.80±14.87	81.13±15.94	59±19.69
250	120.46±13.65	107.00±11.22	87.53±18.49	61.73±18.29
300	72.2±13.11	61.73±18.29	53.26±10.81	29±12.28
350	27.8±5.17	23.40±4.62	17.8±4.59	12.66±2.69
Guava				
100	**235±34.46**	206.80±24.69	184.13±23.09	127.93±23.11
150	184.73±10.32	165.73±17.33	144.26±13.43	107.6±24.81
200	119.2±12.82	114.53±12.88	102.53±16.68	93.46±15.70
250	121.93±8.51	113.73±11.82	101.86±15.67	80.2±14.63
300	99.33±9.15	92.13±10.08	83.563±12.21	70.26±9.40
350	45.66±11.60	39.40±11.67	32.53±9.86	18.93±6.78
Tomato				
100	**218±28.75**	194.53±26.12	169.26±29.74	120.73±24.11
150	154.6±33.09	126.80±24.84	89.73±15.73	34.4±9.01
200	117.06±22.04	77.73±19.57	41.13±16.91	24.86±8.14
250	129.86±15.11	112.60±13.71	90.93±12.28	50.93±15.27
300	62.6±9.89	56.13±8.64	44.40±5.67	25.33±4.27
350	29.66±6.33	25.67±5.64	20.60±4.94	13.86±3.27

Due to hours- $F_{(cal)5\%}$=51.196 > $F_{(tab)5\%}$=2.75, S.E=3.486, C.D at 5%= 7.055(S)
Due to concentration- $F_{(cal)5\%}$=171.696 > $F_{(tab)5\%}$=2.52, S.E=6.971, C.D at 5%=14.109(S)
Due to plants - $F_{(cal)5\%}$=19.8043 > $F_{(tab)5\%}$=2.52, S.E= 4.500, C.D at 5%=9.108(S)
The values are mean of 15 replicates ± SD.

Table 2: Incidence of Methyl Parathion degrading bacterial isolates in rhizospheric soil of selected plants.

Sample size	No.of isolates positive for Methyl Parathion degradation	Percent incidence of Bacterial Isolates		
		P. aeruginosa	***B. megaterium***	***S. aureus***
45	306	119 (39%)	149 (49%)	38 (12%)

$\chi^2_{(cal)=64.64} > \chi^2_{(tab)=7.815}$; S= significant

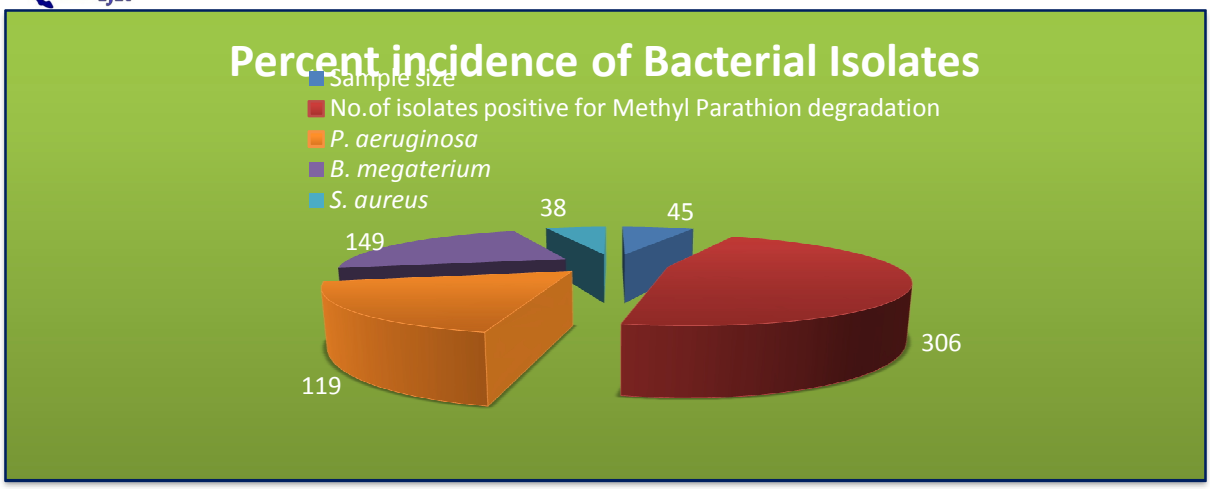

Fig 1: Incidence of Methyl Parathion degrading bacterial isolates in rhizospheric soil of selected plants.

Table 3: Distribution of bacteria in rhizospheric soil w.r.t. selected plants amended with Methyl Parathion.

Sample sites	Sample size(n)	No. of isolates positive for Methyl Parathion degradation	Bacteria isolated from rhizospheric soil amended with Methyl Parathion		
			P. aeruginosa	***B. megaterium***	***S. aureus***
Research Farm, SHIATS, Allahabad (Cabbage)	15	109	34 (31.1%)	**62 (57%)**	13 (12%)
Commercial Farm, Jhunsi, Allahabad (Tomato)	15	92	**54 (58.6%)**	28 (30.4%)	10 (11%)
Guava Orchard, SHIATS, Allahabad (Guava)	15	105	31 (29.5%)	**59 (56.1%)**	15 (14.2%)

Due to sites- $F_{(cal)5\%}$=3.5< $F_{(tab)5\%}$=4.76, S.E=13.042, C.D at 5% = 27.049(NS)
Due to organism-$F_{(cal)5\%}$= 3.23< $F_{(tab)5\%}$=5.14, S.E=13.042, C.D at 5% = 27.049(NS)

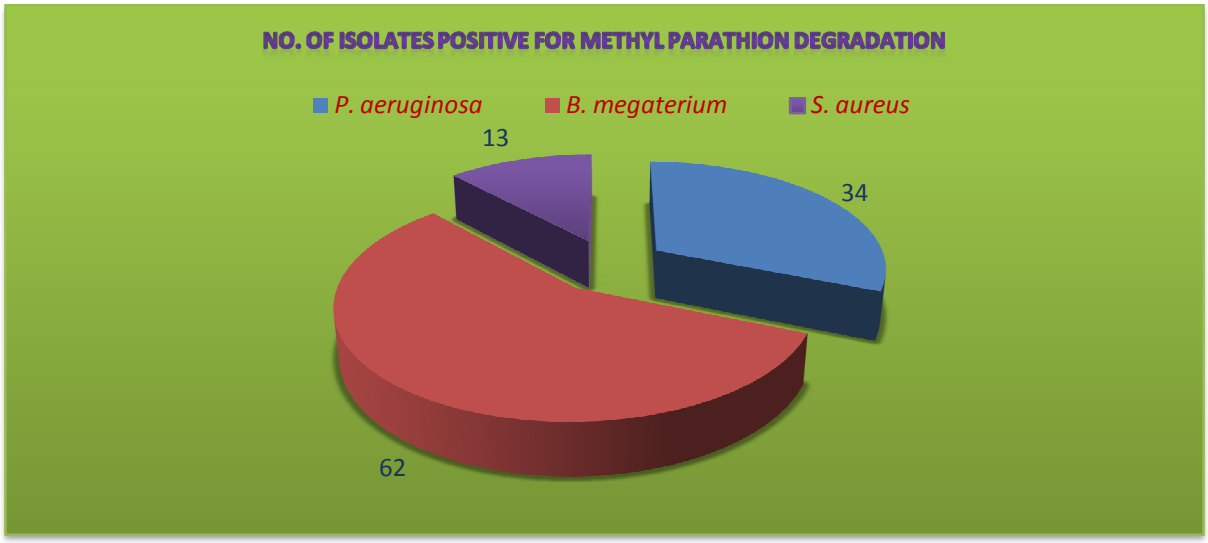

Fig 2: Bacteria isolated from rhizospheric soil amended with Methyl Parathion from Research Farm, SHIATS, Allahabad (Cabbage)

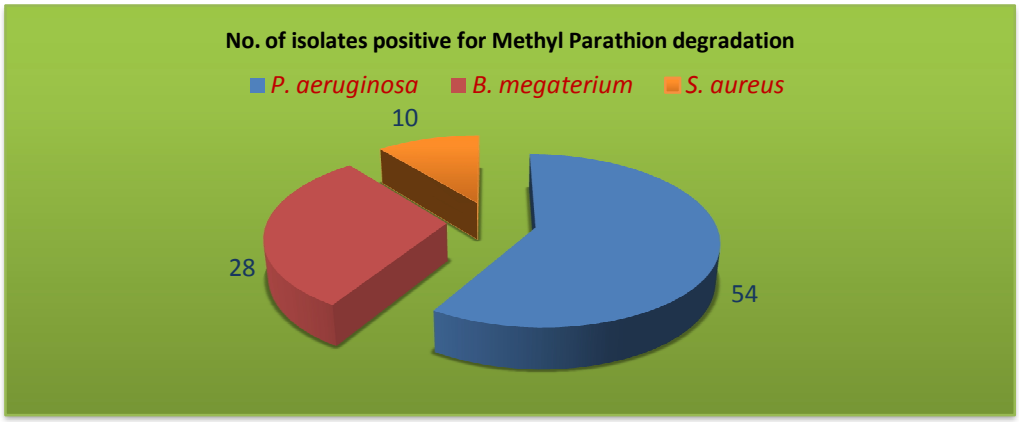

Fig 3: Bacteria isolated from rhizospheric soil amended with Methyl Parathion from Commercial Farm, Jhunsi, Allahabad (Tomato)

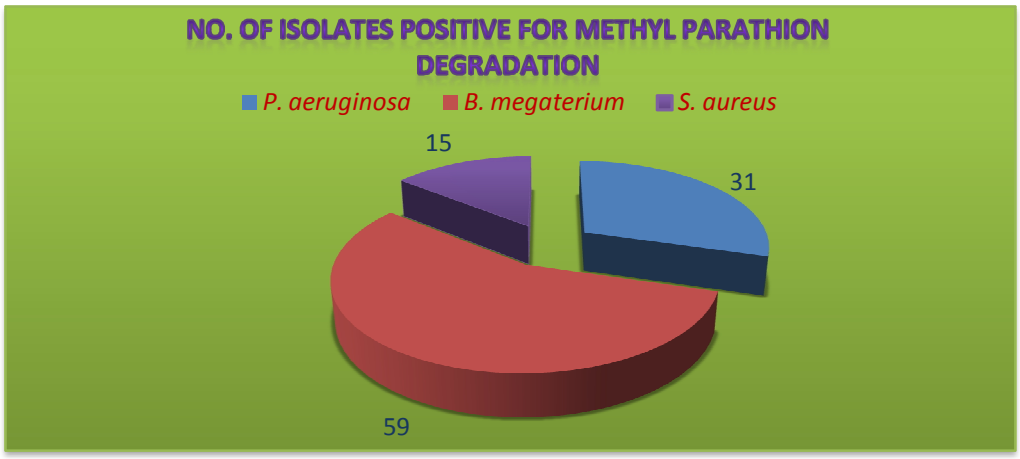

Fig 4: Bacteria isolated from rhizospheric soil amended with Methyl Parathion from Guava Orchard, SHIATS, Allahabad (Guava)

Optimization of the growth condition of Methyl Parathion degrading isolates

Normally, the pH and temperature influence the growth of microorganisms and hence, these factors will influence also the degradation process of the pesticides.

Effect of pH and different concentration on growth of Methyl parathion degrading bacteria

The optimum pH for the growth of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus megaterium* isolated from the rhizospheric soil amended with Methyl Parathion was observed as 7. However, the isolates grew at quite wide range of pH from 3 to 9. This variation is very useful as they can tolerate the pH change during the degradation process and thus increase the degradation potential for these isolates.

Table 4: Effect of pH and different concentrations of Methyl Parathion on growth of *Pseudomonas aeruginosa* (10^4 log cfu/ml).

Methyl Parathion concentration (µg/ml)	*Pseudomonas aeruginosa* (10^4 log cfu/ml)						
	3	**4**	**5**	**6**	**7**	**8**	**9**
100	1.748	1.806	1.903	2.00	**2.113**	1.982	1.939
150	1.698	1.770	1.924	2.017	2.060	1.954	1.903
200	1.707	1.755	1.897	1.986	2.079	1.908	1.880
250	1.643	1.698	1.875	1.963	2.045	1.880	1.875
300	1.556	1.653	1.778	1.944	2.017	1.724	1.732
350	1.518	1.505	1.785	1.851	1.995	1.698	1.698

Due to pH- $F_{(cal)5\%}$= 93.36> $F_{(tab)5\%}$= 2.42, S.E= 0.019, C.D at 5%= 0.039(S)
Due to concentration- $F_{(cal)5\%}$=36.22> $F_{(tab)5\%}$= 2.53, S.E= 0.019, C.D at 5% = 0.039(S)

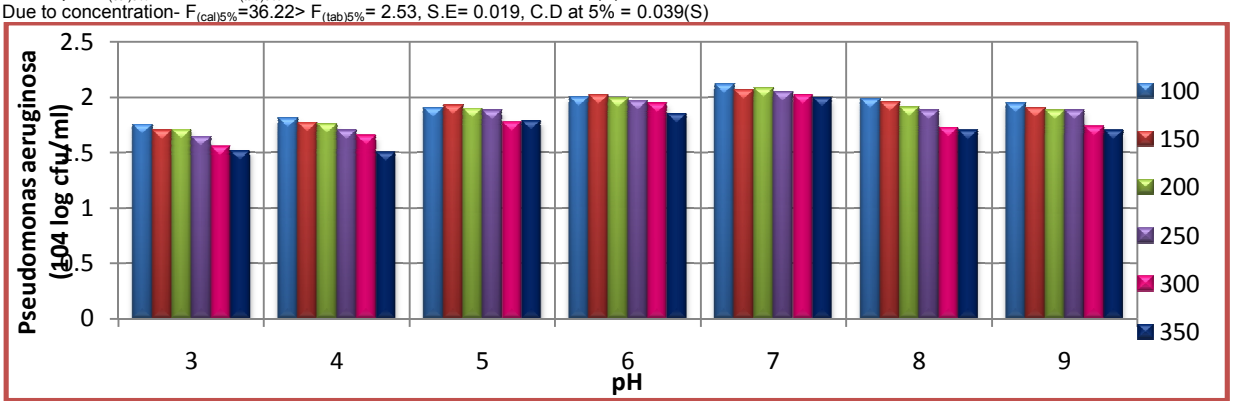

Fig. 5: Effect of pH at different concentrations of Methyl Parathion on growth of *Pseudomonas aeruginosa* (10^4 log cfu/ml)

Table 5: Effect of pH at different concentrations of Methyl Parathion on growth of *Staphylococcus aureus* (10^4 log cfu/ml)

Methyl Parathion concentration (µg/ml)	*Staphylococcus aureus* (10^4 log cfu/ml)						
	3	**4**	**5**	**6**	**7**	**8**	**9**
100	1.579	1.755	**1.929**	2.045	**2.075**	**1.939**	**1.812**
150	1.556	1.740	1.908	**1.986**	1.991	1.892	1.785
200	**1.591**	**1.707**	1.857	1.954	1.929	1.778	1.653
250	1.491	1.690	1.819	1.939	1.886	1.740	1.602
300	1.477	1.653	1.785	1.869	1.869	1.707	1.518
350	1.342	1.518	1.740	1.819	1.845	1.643	1.462

Due to pH- $F_{(cal)5\%}$= 116.74> $F_{(tab)5\%}$=2.42, S.E= 0.019, C.D at 5%= 0.040(S)
Due to concentration- $F_{(cal)5\%}$= 45.58> $F_{(tab)5\%}$=2.53, S.E=0.019, C.D at 5%= 0.040(S)

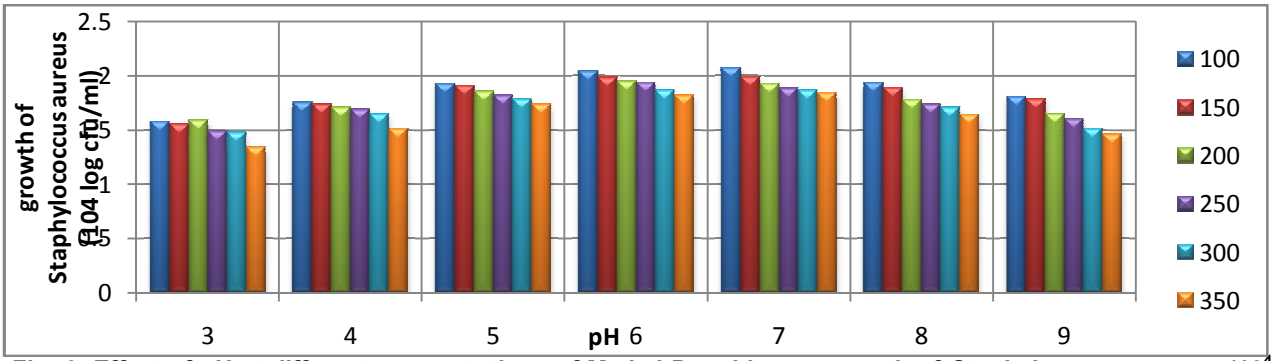

Fig. 6: Effect of pH at different concentrations of Methyl Parathion on growth of *Staphylococcus aureus* (10^4 log cfu/ml)

Table 6: Effect of pH at different concentrations of Methyl Parathion on growth of *Bacillus megaterium* (10^4 log cfu/ml)

Methyl Parathion concentration (µg/ml)	*Bacillus megaterium* (10^4 log cfu/ml)						
	3	**4**	**5**	**6**	**7**	**8**	9
100	**1.653**	**1.897**	**1.977**	**2.082**	**2.178**	**2.00**	**1.973**
150	1.612	1.851	1.954	2.041	2.176	1.963	**1.939**
200	1.568	1.869	1.929	2.017	2.173	1.954	**1.949**
250	1.477	1.838	1.908	1.995	2.100	1.919	**1.826**
300	1.544	1.778	1.897	1.897	2.068	1.869	**1.778**
350	1.477	1.755	1.851	1.845	2.012	1.812	1.732

Due to pH- $F_{(cal)5\%}$= 223.73> $F_{(tab)5\%}$= 2.42, S.E=0.015 , C.D at 5%=0.031(S)
Due to concentration- $F_{(cal)5\%}$=41.73> $F_{(tab)5\%}$=2.53, S.E= 0.015, C.D at 5%=0.031(S)

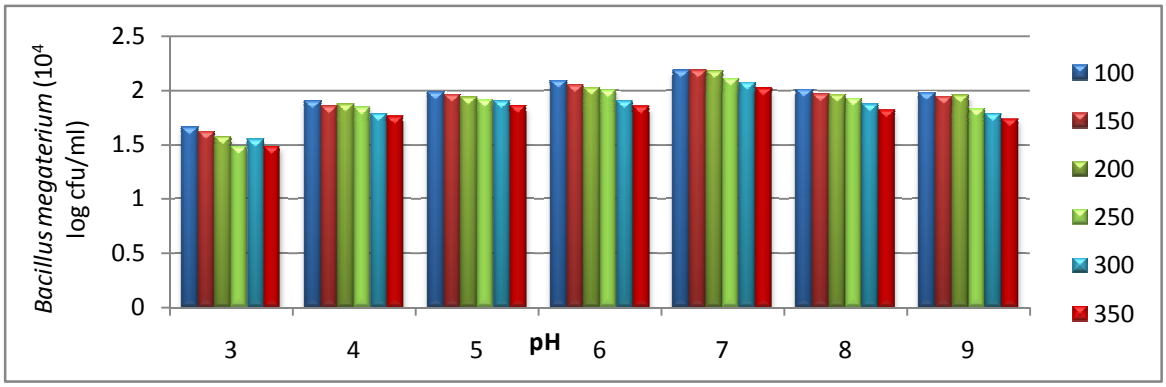

Fig. 7: Effect of pH at different concentrations of Methyl Parathion on growth of *Bacillus megaterium* (10^4 log cfu/ml)

Effect of temperature at different concentrations of Methyl Parathion on growth of isolates

The optimum temperature for the growth of Methyl Parathion degrading isolates identified during the present investigation was observed as 30˚C. However, the growth was observed at a wide range of temperature from 10˚C to 50˚C and was reported in log cfu/ml.

Table 7: Effect of temperature and different concentrations of Methyl Parathion on growth of *Pseudomonas aeruginosa* (10^4 log cfu/ml)

Methyl Parathion concentration (µg/ml)	*Pseudomonas aeruginosa* (10^4 log cfu/ml)				
	10°C	**20°C**	**30°C**	**40°C**	**50°C**
100	**0.602**	**1.397**	**1.949**	1.698	**1.079**
150	**0.602**	1.301	1.903	**1.707**	1.00
200	0.477	1.301	1.886	1.653	1.041
250	0.301	1.255	1.778	1.602	0.093
300	0.301	1.278	1.732	1.491	0.778
350	0.301	1.00	1.602	1.301	0.301

Due to temperature- $F_{(cal)5\%}$=81.47 > $F_{(tab)5\%}$=2.87, S.E= 0.099, C.D at 5%=0.205(S)
Due to concentration- $F_{(cal)5\%}$=6.52 > $F_{(tab)5\%}$=2.71, S.E= 0.099, C.D at 5%=0.205(S)

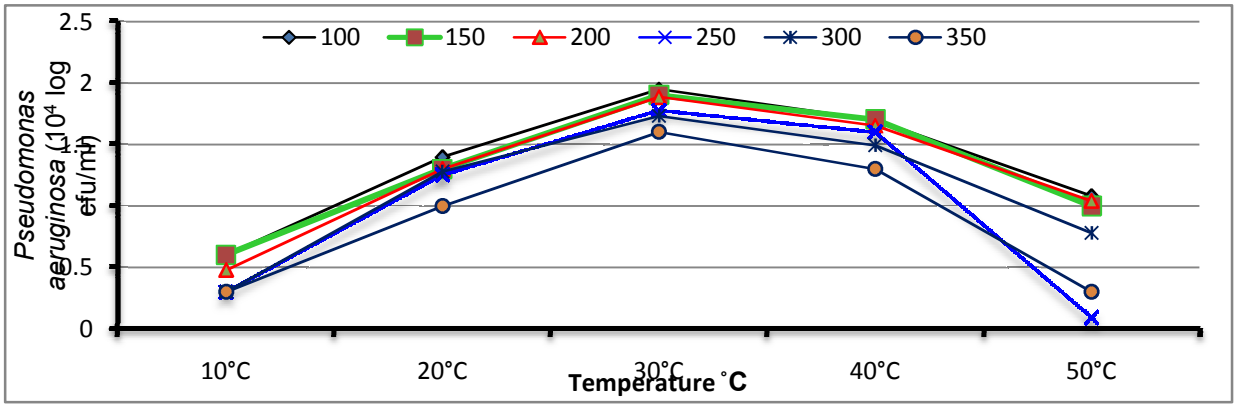

Fig. 8: Effect of temperature at different concentrations of Methyl Parathion on growth of *Pseudomonas aeruginosa* (10^4 log cfu/ml)

Table 8: Effect of temperature at different concentrations of Methyl Parathion on growth of *Staphylococcus aureus* (10^4 log cfu/ml)

Methyl Parathion concentration (µg/ml)	*Staphylococcus aureus* (10^4 log cfu/ml)				
	10°C	**20°C**	**30°C**	**40°C**	**50°C**
100	**1.00**	**1.778**	**2.041**	**1.812**	1.255
150	0.845	1.763	1.954	1.799	**1.176**
200	0.903	1.707	1.949	1.732	1.00
250	0.698	1.662	1.949	1.698	0.954
300	0.301	1.602	1.903	1.643	0.954
350	0.301	1.342	1.869	1.505	0.602

Due to temperature- $F_{(cal)5\%}$=134.26> $F_{(tab)5\%}$=2.87, S.E= 0.071, C.D at 5%= 0.148(S)
Due to concentration- $F_{(cal)5\%}$=10.73 > $F_{(tab)5\%}$= 2.71, S.E= 0.071, C.D at 5%= 0.148(S)

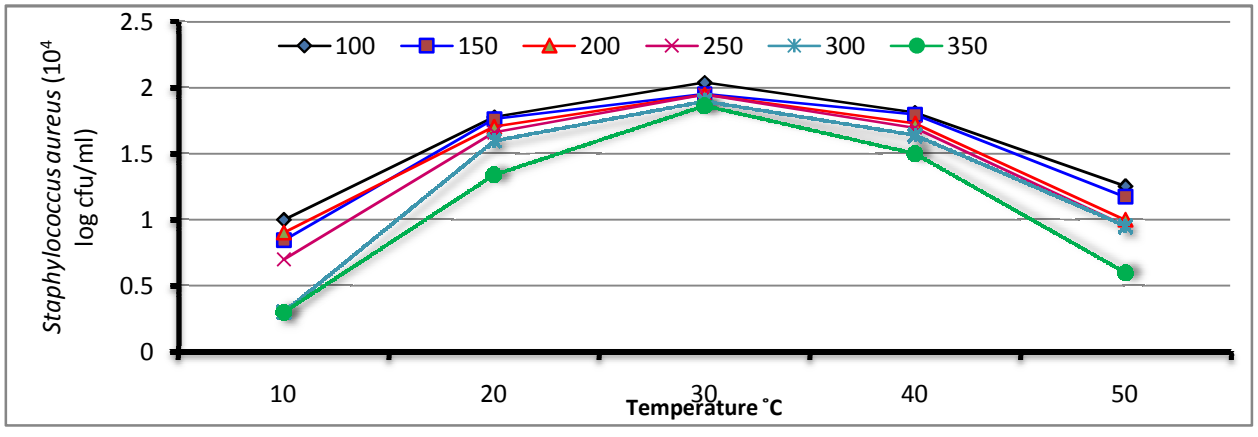

Fig. 9: Effect of temperature at different concentrations of Methyl Parathion on growth of *Staphylococcus aureus* (10^4 log cfu/ml)

Table 9: Effect of temperature at different concentrations of Methyl Parathion on growth of *Bacillus megaterium* (10^4 log cfu/ml)

Methyl Parathion concentration (µg/ml)	*Bacillus megaterium* (10^4 log cfu/ml)				
	10°C	**20°C**	**30°C**	**40°C**	**50°C**
100	**0.778**	1.477	**2.021**	**1.973**	**1.146**
150	**0.778**	**1.491**	2.00	1.954	1.079
200	0.602	1.414	1.977	1.944	0.903
250	0.477	1.397	1.934	1.903	0.903
300	0.301	1.230	1.851	1.851	0.778
350	0.301	1.00	1.778	1.819	0.602

Due to temperature- $F_{(cal)5\%}$=358.93 > $F_{(tab)5\%}$=2.87, S.E= 0.050, C.D at 5%= 0.104(S)
Due to concentration- $F_{(cal)5\%}$=17.51 > $F_{(tab)5\%}$= 2.71, S.E= 0.050, C.D at 5%= 0.104(S)

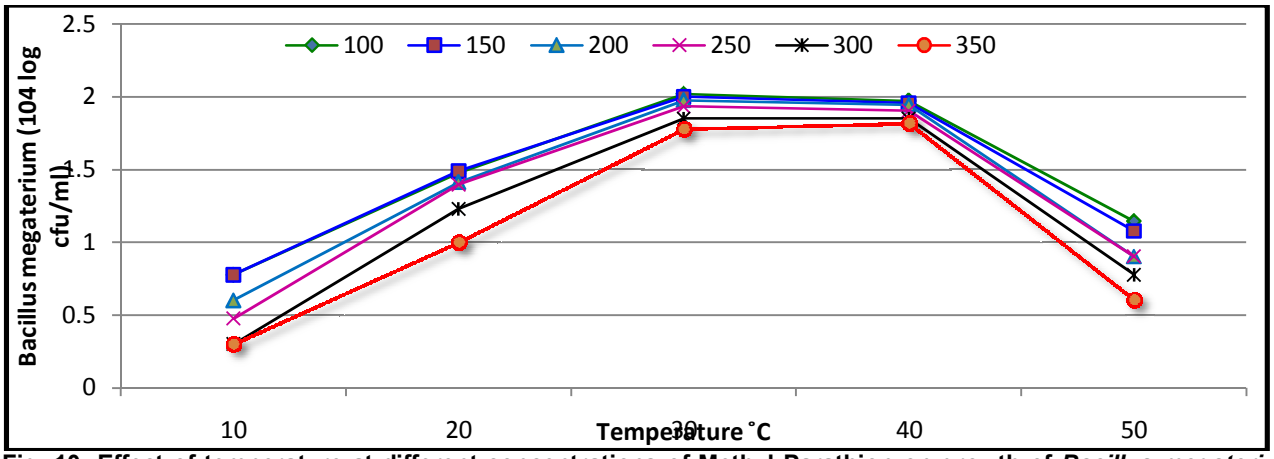

Fig. 10: Effect of temperature at different concentrations of Methyl Parathion on growth of *Bacillus megaterium* (10^4 log cfu/ml)

DISCUSSIONS

Soil environment is characterized by the presence of multiple xenobiotic organic substances. Final destination of these substances in the environment is strongly affected by the nature and concentration of other components in aqueous and solid phases, especially colloids and macromolecules (organic and minerals), which often form complexes with organic contaminants **(Masaphy *et al*,1996).** In this work degradation of Methyl Parathion was studied by bacterial species isolated from agricultural soils and optimization of growth conditions of the bacteria was also studied.

Concerning the microorganisms adaptation process to laboratory conditions and to different concentrations of Methyl Parathion, studied demonstrated that there was a regular decrease in the bacterial load as the concentration of Methyl Parathion was increased which suggests a hydrolysis caused by the enzymatic action of the bacteria group. From the results of this work, we cannot conclude if this hydrolysis takes later on to a complete mineralization (breakdown of the p-Nitrophenol ring) or thio metabolite remains in the medium. On the other hand, the isolated bacteria have been in the presence of some Organophosphate pesticide in the soil, that probably has forced them to generate new enzymes (such as Parathion hydrolase for which no natural substrate is known), and also new metabolic routes for the degradation of this type of organic compound. The environmental conditions, the pH of the soil, the agricultural practices and the quantity of pesticide added in each region, are among other causes, that can be the decisive factor to force bacteria to use xenobiotic compounds (such as pesticides), as substrate for their growth.

.The isolated organisms degrading Methyl Parathion showed maximum growth at pH 7 and 30˚C temperature. This suggests that the enzyme activity of the isolates leading to the hydrolysis of Methyl Parathion was at pH 7 was greater than

that at other pH. The crude enzyme preparations had a broad pH profile and maximum activity was observed at pH 7.5-9.5. Optimum temperature of Methyl Parathion degrading isolates was observed as 30˚C which suggests that the utilization of Methyl Parathion by the resistant microorganisms occurs at 30˚C.

The Organophosphorus hydrolase enzyme which degrades Methyl Parathion to its metabolic product *i.e* p-Nitrophenol might have been more stable at 30˚C.

It can be observed that bacteria isolated from agricultural soils are mainly pathogens **(Palleroni 1984 and Brenner 1984, mentioned by Kreig and Holt 1984, Govan *et al.* 1996)**, and therefore it is very difficult to establish a recommendation for the extensive use of one of these strains in natural environments. Even though a Parathion hydrolase activity was observed, it is important to consider public health risks that could be present. Nevertherless, it is a potential source of enzymes that can reduce the pollution by these pesticides in the environment, using them directly in field or using specific reactors under controlled conditions. For this purpose, research must continue in order to know the nature of the enzyme, its optimal activity characteristics and its life span in natural environments.

CONCLUSION

During the study, it could be concluded that bacterial population was highest at low concentration of Methyl Parathion, whereas it had a declining trend as the concentration of Methyl Parathion was increased and least was found at 350µg/ml concentration of Methyl Parathion. The isolates that could degrade Methyl Parathion were identified as *P. aeruginosa, S. aureus* and *B. megaterium* from rhizosphere of Cabbage, Guava and Tomato. The effect of pH and temperature on growth of bacterial isolates was also observed. The highest growth was observed at pH 7 and 30˚C temperature at 100µg/ml concentration of Methyl Parathion.

Therefore, in the present study, experimental findings indicated that, higher concentration of Methyl Parathion effects the growth of bacterial population and growth, as well as different concentrations has effect on optimum pH and temperature of isolates.

The future aspect of this study involves analysis of various degradation pathways. Further studies are required to explore different enzymes present in microorganisms responsible for Methyl Parathion degradation. The studies could be extended involving monitoring the effect of toxicity of Methyl Parathion on various animals, plants, microorganisms as well as humans.

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